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Interorgan metabolism of amino acids, glucose, lactate, glycerol and uric acid in the domestic fowl (*Gallus domesticus*)

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1. Arterial–venous differences for metabolites across liver, kidney and hindquarters were measured in fed or starved, artificially ventilated chickens. 2. The results indicate that the liver takes up amino acids under both conditions. Urate and glucose are released by the liver in both the fed and the starved state. 3. Lactate and amino acids are extracted from blood by the kidneys, and this increases in the starved chicken. Urate is removed from the circulation by the kidney in the fed and starved state and excreted. In the fed bird there is no significant arteriovenous difference of glucose across the kidney, but in the starved state the kidney releases glucose into the circulation. 4. The hindquarters take up glucose in the fed but not in the starved state. The branched-chain amino acids valine and leucine were taken up by the hindquarters in the fed, but not the starved, chicken. Glycerol is released by the hindquarter of fed and starved chickens. In the starved state, alanine and glutamine represent 57% of the amino acids released by the hindquarter. Lactate is released by the hindquarter of starved chickens and represents the major gluconeogenic carbon source released by the hindquarter and taken up by kidney and liver. 5. Although the liver is the major gluconeogenic organ in the starved chicken, the kidney accounts for approx. 30% of the glucose produced.

INTRODUCTION

Interest in gluconeogenesis from amino acids in the chicken has led various investigators to examine the concentrations of amino acids in the blood of that species (Boomgaardt & MacDonald, 1969; Brady *et al*, 1978; Hill & Olsen, 1963; Zimmerman & Scott, 1967). The conclusions that can be drawn from measurements of metabolites or amino acids in plasma are limited, however. A change in the plasma concentration of a substance can be caused by a change in production, utilization or excretion. Measurement or arteriovenous differences across an organ permits more definitive conclusions to be made about the net metabolism of that organ. Although glucose arteriovenous differences have been measured across the wing of the chicken (Knapp, 1936), there has been no report of amino acid or other metabolite arteriovenous-difference measurements in birds *in vivo*. This may be due to the difficulties of sampling from abdominal vessels without causing the bird to become hypoxic. The refinement of a technique for maintaining blood gases of abdominally opened chickens, at physiological values, has allowed the measurement of arteriovenous differences across liver, hindquarter and kidney in the fowl for the first time. The results of these measurements are reported and discussed in this paper. An abstract of this work has been presented (Tinker *et al.*, 1982).

EXPERIMENTAL

Animals

Fertilized eggs were obtained from a flock of White Leghorn chickens (*Gallus domesticus*) maintained at Memorial University's Animal Care Facilities. Newly hatched chicks were reared at 25 °C with room lighting on 24 h daily. Water and commercial chick starter feed (Supersweet Feeds, St. John's, Newfoundland, Canada; 20% protein, 2% fat, 6% fibre) were available *ad libitum*. Experimental animals were males taken from the facility at 8 weeks of age, at which time they had a mass of 800 g. The animals were either used immediately or starved in wire-bottom cages for 6 days before use. Water was available *ad libitum* to the starved animals.

Assay methods

Amino acids. Amino acids were determined on a Beckman model 121M amino acid analyser and Beckman Systems AA Computing Integrator by using a five-buffer single-column method as described by Lee (1974) and modified in Beckman Bulletin 12 IM TB-013 (July 1976).

Metabolites. Glucose was determined enzymically by using a coupled hexakinase glucose-6-phosphate dehydrogenase assay (Raabo & Terkildsen, 1960). Ammonia was determined enzymically by using glutamate dehydrogenase (Kun & Kearney, 1974). Lactate was determined enzymically with lactate dehydrogenase (Lowry & Passonneau, 1972). Glycerol was determined enzymically by using a coupled glycerokinase/glycerol-3-phosphate dehydrogenase assay (Wieland, 1974). Uric acid was determined with uricase as described in Sigma Technical Bulletin 292-UV with reagents obtained in kit form from Sigma Chemical Co., St. Louis, MO, U.S.A. Hepatic blood flow was determined with bromosulphophthalein (Bradley *et al.*, 1945).

Artificial ventilation and blood sampling

Surgical techniques were previously described in detail by Tinker *et al.* (1984*a*). Briefly, cockerels were

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anaesthetized by an intravenous (wing vein) dose of sodium pentobarbital (approx 40 mg/kg). The trachea of the animal was exposed and cut through; 3 cm of stiff plastic tubing was inserted into the trachea, ligated and attached in series to a gas humidifier, a flowmeter and gas regulator. The abdominal cavity was opened by a cut beneath the sternum, and humidified air was passed through the trachea at a flow rate of 450–600 ml/min. This technique results in normal blood gases and pH (pO_2, pCO_2 and pH were 58 mmHg, 28 mmHg and 7.40 in conscious birds and 54 mmHg, 32 mmHg and 7.36 in ventilated birds respectively), as well as more physiological hepatic metabolite contents (ATP = 1.88 μmol/g, ATP/ADP = 1.37, lactate = 0.52 μmol/g; Tinker *et al.*, 1984*a*).

For blood lactate, ammonia and amino acid determinations, five 2 ml blood samples were taken with heparinized syringes from ventilated birds. The sampling sites were the left hepatic vein (1 cm before its entry into the caudal vena cava), the right hepatic portal vein (1 cm before its entry into the margin of the liver), the left external iliac vein (1 cm before its branches into the common iliac and caudal renal portal vein), the left caudal renal vein (1 cm before its entry into the common iliac vein) and the inferior aorta (at the level of the branching-off of the femoral arteries). The vessels were sampled in that order over a time period of 5 min. After removal of the blood samples, the animals was humanely killed by an intra-cardiac overdose of sodium pentobarbital.

Liver arterial–venous differences for glucose, urate and glycerol were determined in separate birds surgically prepared as above. Blood samples were taken from the inferior aorta, hepatic vein and hepatic portal vein. Kidney and hindquarter arterial–venous differences were determined in separate birds prepared as above. Blood samples were taken from the inferior aorta, external iliac vein and renal vein. These blood samples were divided into two 0.5 ml subsamples and treated as follows: (1) For amino acids in whole blood, a 0.5 ml blood subsample was deproteinized with 1 ml of cold 10% (w/v) sulphosalicyclic acid. This was mixed and centrifuged at 20000 *g* for 20 min. The supernatant was collected and its pH adjusted to 2.2 with 3 mM-LiOH. An amount of 0.15 M-lithium citrate buffer (pH 2.2) equal to half the volume of the neutralized supernatant was added, and the sample stored frozen (−40 °C) before analysis for amino acids. (2) For whole blood glucose, lactate, urate, glycerol and ammonia, the second 0.5 ml blood sample was deproteinized with 1 ml of cold 6% (w/v) $HClO_4$. Precipitated protein was removed by centrifugation at 20000 *g* for 20 min. The pH of the supernatant was adjusted to 7.0 with KOH to precipitate $KClO_4$, and the supernatant centrifuged at 2000 *g* for 10 min. The second supernatant was analysed for glucose, lactate, urate, ammonia and glycerol.

The stability of the preparation during blood sampling was investigated. There was no difference in the haematocrits of the five blood samples taken. There was no significant change in haematocrit in the cockerels after 6 days of starvation. In a separate experiment on anaesthetized ventilated cockerels (results not shown), 2 ml blood samples were drawn from the inferior aorta through an indwelling cannula at 2 min intervals for 12 min. There was no significant effect of sampling on haematocrit or on glucose in the arterial blood. Thus the steady state was not disturbed during the course of the experiment.

Calculations

Arteriovenous differences for hindquarter were calculated by subtracting the concentration of the metabolite in the external iliac vein from the concentration in the inferior aorta. The afferent–efferent differences for liver were calculated by assuming that the arterial blood perfusing the liver comprised 30% of the total (Sapirstein & Hartman, 1959). The afferent–efferent differences for the kidney were calculated by assuming that the arterial component of renal flow comprised 50% of total renal flow, with the renal portal veins supplying the balance (Skadhauge, 1973). It was assumed that the concentrations of metabolites in the caudal renal portal vein were equal to those in the external iliac vein, and that blood in the caudal renal portal vein flows into the kidney. Other assumptions that were necessary for these calculations were that (1) the concentrations of metabolites in all arteries were equal to those in the inferior aorta, (2) blood in the hepatic portal vein flows towards the liver and that concentrations of metabolites were equal in both left and right portal veins, (3) concentrations of metabolites in all efferent hepatic veins were assumed to be equal to those in the left hepatic vein and (4) the same patterns of flow exist in both fed and starved chickens.

Glucose, glycogen, and pyruvate dehydrogenase activity

In separate experiments, freeze-clamped samples of liver, kidney and gastrocnemius muscle were obtained from artificially ventilated fed and 6-day-starved chickens. One portion of freeze-clamped tissue was used to determine glucose and glycogen as previously described (Tinker *et al.*, 1984*a*). A second portion of freeze-clamped tissue was homogenized in 10 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA, 1 mM-dithiothreitol and bovine serum albumin (10 g/l). One portion of the homogenate was analysed immediately for pyruvate dehydrogenase activity (the active portion). A second portion was incubated for 30 min at 30 °C in the presence of 10 mM-$MgCl_2$ to activate pyruvate dehydrogenase fully and then analysed for the enzyme's activity (Walajtys *et al.*, 1974). Pyruvate dehydrogenase activity was determined from the rate of decarboxylation of [1-^{14}C]pyruvate (Taylor *et al.*, 1973).

RESULTS

Blood flow

Hepatic blood flow decreased in the starved chicken from 59.0±0.9 ml/min/kg body wt. (fed) to 36.0±6.1 (starved). However, since the liver decreases in weight during starvation from 3.08±0.21 g/100 g body wt (fed) to 1.92±0.17 (starved), the flow rate per g of liver was unchanged (1.92±0.03 ml/min per g of liver, fed; 1.92±0.53, starved).

Arterial metabolite concentrations

Amino acid and metabolite concentrations in arterial blood from fed and starved chickens are presented in Table 1. The values are similar to those reported by other investigators (Boomgaardt & MacDonald, 1969; Brady *et al.*, 1978; Hill & Olsen, 1963; Zimmerman & Scott, 1967). Blood from starved chickens had significantly

Table 1. Arterial whole blood concentrations of amino acids and metabolites in chickens

Values marked with an asterisk are significantly different from the corresponding fed values ($P < 0.05$, Student's t test). Values are means ± S.E.M. Chickens were starved for 6 days. In the fed state, $n = 6$ for amino acids, $n = 7$ for glucose, urate, glycerol and lactate. In the starved state, $n = 5$ for amino acids, $n = 7$ for glucose, urate, and glycerol, and $n = 11$ for lactate.

	Concn. (nmol/ml of whole blood)	
	Fed	Starved
Taurine	3550±98	3850±160
Hydroxyproline	154±18	69±6*
Threonine	329±21	634±124*
Serine	581±64	437±31
Asparagine	68±9	45±4
Glutamate	740±47	384±25*
Glutamine	444±67	370±31
Proline	214±17	149±11*
Glycine	606±33	538±26
Alanine	518±40	323±14*
Valine	336±30	519±76
Cysteine	98±6	53±2*
Methionine	14±9	67±7*
Isoleucine	138±18	173±24
Leucine	244±28	236±28
Tyrosine	148±12	106±6*
Phenylalanine	93±5	84±10
β-Alanine	403±22	361±33
Tryptophan	29±2	31±4
Ornithine	21±4	49±10*
Lysine	312±68	239±30
Histidine	65±5	60±7
3-Methylhistidine	15±2	48±8*
Anserine	596±71	695±70
Carnosine	1640±147	1310±245
Arginine	258±25	158±14*
Glucose	10360±231	11670±665
Glycerol	190±18	183±13
Lactate	2340±290	2360±170
Urate	212±20	233±29

elevated concentrations of threonine, methionine, ornithine and 3-methylhistidine and significantly lower concentrations of hydroxyproline, glutamate, proline, alanine, cysteine, tyrosine and arginine. Blood glucose concentrations were maintained during the 6 days of starvation.

Metabolism of amino acids and other metabolites

Arterial–venous differences across liver, kidney and hindquarter for fed and starved chickens are presented in Table 2.

Liver

In the fed chicken, there was significant hepatic uptake of asparagine, glutamate, proline, isoleucine, leucine, phenylalanine, tryptophan, arginine and lactate. There was significant release of urate and glucose. Liver of starved chicken removed significant amounts of threonine, serine, glutamine, glycine, alanine, tyrosine, phenylalanine, ornithine, lysine, arginine and lactate, and released significant quantities of urate and glucose. The number and quantity of amino acids removed by the liver increased in the starved state (fed, 188±58 nmol/ml; starved 665±160 nmol/ml; $P < 0.01$).

Kidney

There was significant removal of hydroxyproline, glutamine, alanine, glycerol and urate by kidney of fed chickens. The kidney of starved chickens removed glycine, alanine, phenylalanine, cysteine, carnosine, arginine, glycerol, urate and lactate, and released serine, ammonia and glucose.

Hindquarter

There was significant uptake of proline, valine, leucine and glucose by hindquarter of fed chicken and release of glycerol and urate. In the starved state, there was significant uptake of ammonia, and release of glutamine, glycine, alanine, methionine, tyrosine, phenylalanine, lysine, histidine, glycerol, urate and lactate. There was a large increase in the number and quantity of amino acids released by hindquarter of starved compared with fed chickens (no amino acids released in the fed state, eight amino acids, totalling 408±98 nmol/nl in the starved state; $P < 0.001$). The significant release of glycerol in the fed and starved states is likely to be due to the contribution of adipose tissue to the hindquarter arteriovenous difference. Alanine and glutamine accounted for 57% of the amino acids released by hindquarter. Alanine account for 31% of the amino acids released, but only 5.6% of the amino acid content of chicken leg muscle (Food and Agricultural Organization, 1970). Release of these amino acids in the proportions observed must therefore involve interconversion of amino acids in the muscle, as Felig (1975) has suggested for humans.

Tissue glycogen and glucose contents

Kidney glycogen was very low (Table 3) and unaffected by starvation. Kidney glucose was also unaffected by starvation. Muscle glycogen was decreased by about 50% by starvation. This is a more extensive decrease than reported by Riesenfeld *et al.* (1981) for chicken pectoralis muscle, where it was unchanged for 4 days of starvation. Muscle glucose was low compared with other tissues examined, and was decreased by about 20% by starvation. Liver glycogen was significantly higher than reported by Bobyleva-Guarriero *et al.* (1984) in both fed and starved chickens. This is presumably due to glycogenolysis during their freeze-clamping procedure, since they also found hepatic glucose contents 2–4 times those that we have previously reported for anoxic chicken liver (Tinker *et al.*, 1984*a*). We observed a decrease of over 50% in hepatic glucose in 6-day-starved chickens.

Pyruvate dehydrogenase activity

Of the three tissues examined, liver had the highest total pyruvate dehydrogenase activity and muscle the lowest (Table 4). Total activity was unaltered by starvation in muscle and liver, but was decreased nearly by half in kidney. In both muscle and liver, the enzyme was 90% or more in the active state in fed chickens; this was decreased to 10% or less by starvation. In the kidney, the enzyme was only 58% active in the fed state and was only decreased to 34% active by starvation.

Table 2. Arteriovenous differences for amino acids and other metabolites across liver, kidney and hindquarter of fed and starved chickens

Differences across liver and kidney are shown for the assumptions that the arterial component of tissue flow is 30% and 50% respectively. Values marked with an asterisk are significantly different from zero ($P < 0.05$, paired t test). Values are means ± S.E.M. Chickens were starved for 6 days. In the fed state $n = 6$ for amino acids and peptides, $n = 7$ for glucose, uric acid, glycerol and lactate. In the starved state, $n = 5$ for amino acids and peptides, $n = 7$ for glucose, uric acid, glycerol and $n = 11$ for lactate. Metabolites were measured in whole blood. A positive sign indicates an uptake and a negative sign a release.

	Arteriovenous difference (nmol/ml of whole blood)					
	Liver		Kidney		Hindquarter	
Metabolite	Fed	Starved	Fed	Starved	Fed	Starved
Taurine	186±140	229±258	581±345	370±204	117±163	−99±340
Hydroxyproline	−18±12	15±7	29±9*	−10±18	−38±18	−2±13
Threonine	23±12	75±14*	14±13	28±17	16±13	−25±46
Serine	41±23	112±10*	−57±55	−78±22*	55±40	−26±33
Asparagine	9±3*	16±8	2±4	1±4	−4±6	−12±6
Glutamate	76±26*	35±16	−30±43	13±12	86±43	0±15
Glutamine	6±28	121±28*	62±20*	33±17	−48±30	−109±17*
Proline	21±5*	67±29	10±8	−2±10	24±5*	−14±10
Glycine	64±27	85±15*	21±22	52±13*	25±22	−75±17*
Alanine	62±44	128±17*	58±11*	75±19*	−10±30	−125±20*
Valine	22±10	93±44	24±13	40±30	25±5*	−7±20
Methionine	−2±7	10±5	−8±10	2±4	2±12	−25±3*
Isoleucine	15±4*	22±13	4±5	11±8	10±5	−7±8
Leucine	26±6*	33±16	10±7	13±6	18±7*	−11±6
Tyrosine	14±6	28±7*	3±6	5±2	8±8	−11±3*
Phenylalanine	14±5*	25±5*	1±3	6±2*	8±4	−8±3*
β-Alanine	17±14	6±61	24±20	−37±31	16±40	−81±64
Tryptophan	3±1*	−1±2	3±3	4±3	−2±2	−5±3
Ornithine	1±1	12±5*	4±2	−1±4	−3±2	−1±3
Lysine	33±15	57±18*	−1±13	1±6	6±12	−39±13*
Histidine	7±3	12±19	−1±3	−14±10	−2±5	−16±4*
Cysteine	19±8	6±5	−4±6	9±1*	−1±6	7±3
3-Methylhistidine	1±1	5±4	1±1	0±1	1±1	0±2
Anserine	9±19	29±49	32±30	67±32	41±38	−77±36
Carnosine	68±60	111±121	69±73	120±37*	76±91	−46±28
Arginine	24±10*	22±8*	4±11	34±10*	13±12	−36±16
Glycerol	21±17	39±22	16±5*	31±7*	−36±12*	−47±9*
Ammonia	34±33	62±52	−58±23	−123±44*	43±55	41±16*
Lactate	523±199*	1010±130*	95±222	356±121*	−233±392	−441±111*
Glucose	−940±272*	−1450±359*	−44±83	−307±136*	792±126*	199±108
Urate	−100±17*	−76±22*	84±14*	83±17*	−72±23*	−50±13*

DISCUSSION

This paper presents, for the first time, a comprehensive study of the interorgan metabolism of amino acids, glucose and other metabolic fuels in the intact chicken. Because this is the first time that this ventilation technique has been used for metabolic studies in birds, thee are no comparable data available on metabolites in the abdominal vessels. A limited amount of work has been carried out on muscle metabolism by using wing veins, which can be reliably sampled without the use of this ventilation technique. Knapp (1936) has shown that there was an uptake of glucose across the wings of both roosters and hens. Other metabolites were not measured. In a classic study Cori & Cori (1925) demonstrated that blood drawn from a chicken wing vein with a Rous sarcoma contained more lactate and less glucose than did blood drawn from the contralateral wing vein.

The fed chicken

In the fed chicken, the major metabolite fluxes were across the liver. A number of amino acids, including the essential amino acids arginine, isoleucine, leucine and phenylalanine, were removed by the liver. In addition glutamine, glutamate, asparagine and proline were taken up. Significant amounts of lactate were also removed by the liver. The amino acids were presumably supplied by the diet, since neither kidney nor hindquarter released any amino acids to the circulation. Urate was produced by the liver. The removal of glutamine and asparagine by the liver is consistent with the production of urate, since two of the nitrogen atoms of urate come from glutamine and the third comes from aspartate, which could be generated from asparagine (Mapes & Krebs, 1978). Data on aspartate fluxes are not available, because the aspartate peak is masked by glutathione in the amino acid chromatography.

There was a significant release of glucose by the liver. The possibility exists that the glucose release observed from liver of fed and starved chickens is the result of glycogenolysis from the stress of the surgical procedures (Freeman, 1971; Freeman & Manning, 1976). To examine this possibility, liver and kidney were rapidly

Table 3. Glycogen and glucose in gastrocnemius muscle, kidney and liver of fed and starved chickens

Tissues were freeze clamped in birds that had been artificially ventilated as described in the text. Results are means ± S.D. from four fed and four starved birds. Chickens were starved for 6 days. Values are expressed as μmol of glucose or glucose equivalents/g wet wt.* Value significantly different from the fed value ($P < 0.001$) by Student's *t* test.

	Glycogen		Glucose	
	Fed	Starved	Fed	Starved
Muscle	54.4±4.8	25.8±6.8*	3.6±0.3	2.9±0.4*
Kidney	2.8±2.1	1.3±0.5	8.7±1.6	9.1±0.9
Liver	86.4±16.0	18.5±3.1*	9.3±0.6	4.2±0.2*

Table 4. Pyruvate dehydrogenase activity in gastrocnemius muscle, kidney and liver of fed and starved chickens

Tissues were freeze-clamped in artificially ventilated birds, and homogenates were prepared as described in the text. Enzyme activity was determined before and after activation in the presence of $MgCl_2$. The ratio was used to calculate the percentage of the enzyme in the active form *in vivo*. Total activity is the activity after activation in the presence of $MgCl_2$, and is expressed as μmol of pyruvate decarboxylated/min per g of tissue. Values are means ± S.D. for four fed and four starved chickens. Chickens were starved for 6 days. * Value significantly different from the fed value ($P < 0.01$) by Student's *t* test.

	Pyruvate dehydrogenase activity			
	Fed		Starved	
	Total	% Active	Total	% Active
Muscle	0.35±0.04	89.0±29.8	0.36±0.25	1.1±1.0*
Kidney	1.89±0.42	57.9±9.0	1.01±0.09*	33.8±17.3*
Liver	4.47±0.40	91.0±34	4.50±0.40	10.0±2.6*

freeze-clamped in unventilated chickens to see whether or not glycogen and glucose were different between chickens *in vivo* and those being artificially ventilated. The values obtained (μmol of glucose equivalents/g; means ± S.D., $n = 3$) were not significantly different from those obtained in ventilated birds [glycogen: liver (fed), 80.7±13.3; liver (starved), 15.0±4.3; kidney (starved), 1.6±0.6; glucose liver (fed), 10.0±0.7; liver (starved), 4.0±0.3; kidney (starved), 8.9±1.0]. The glycogen values were not different from those in ventilated birds, and the glucose values, which would be expected to increase if glycogenolysis were occurring, was also unchanged by the ventilation technique. Thus we believe the observed release of glucose is a result of gluconeogenesis, not glycogenolysis.

The glucose released by the liver in the fed state probably reflects gluconeogenesis from intestinally produced lactate. Riesenfeld *et al.* (1982) have shown that a significant portion (up to 37%) of absorbed glucose is converted into lactate by the intestinal tract. It is possible that some of this lactate is reconverted into glucose by the liver in the fed state.

Glucose is removed by the hindquarter and presumably oxidized, since there is no release of lactate by the hindquarter in fed chickens. This is supported by the high activity state of pyruvate dehydrogenase in muscle in fed chickens (Table 4). Leucine, valine and proline are also removed by hindquarter of the fed chicken, which also released glycerol, presumably as a result of triacylglycerol catabolism in the fed chicken. Both adipose tissue and muscle have lipoprotein lipase, which releases glycerol during the catabolism of chylomicron triacylglycerol (Cryer, 1981).

Unexpectedly, the hindquarter releases significant amounts of urate. It seems unlikely that muscle or adipose tissue is capable of uric acid synthesis *de novo*. However, it is possible that liver and/or kidney or intestine release hypoxanthine to the circulation, which might be reduced to urate by xanthine dehydrogenase in hindquarter. It has previously been shown in the pigeon that an interogan flux of hypoxanthine is involved in uric acid synthesis (Edson *et al.*, 1936). Pigeon liver lacks xanthine dehydrogenase. In this species hypoxanthine is produced in liver and transported to the kidney, where it is oxidized to uric acid by kidney xanthine dehydrogenase. Morgan (1926) did not find xanthine dehydrogenase in chicken muscle; however, he also did not find activity in intestine or pancreas. Remy & Westerfield (1951) later demonstrated the presence of xanthine dehydrogenase in both pancreas and intestine, but did not examine muscle. Our observation of urate release by hindquarter suggests that muscle should be examined for xanthine dehydrogenase activity.

The data in Tables 1 and 2 show that the kidneys removed about one-third to one-half of the blood urate presented to them in each pass. This is much more than could be accounted for by glomerular filtration alone, since the filtration fraction in chicken kidneys is only about one-tenth of the renal blood flow (Sykes, 1971). Thus our renal arteriovenous data necessitate urate elimination by means of tubular secretion. Such a mechanism has already been proposed by several workers on the basis of clearance experiments (Berger *et al.*, 1960; Nechay & Nechay, 1959; Shannon, 1938). The renal removal of urate supports our assumptions about renal blood flow and suggest that our calculated renal arteriovenous differences are physiologically meaningful.

Both liver and kidneys have the capacity for urate synthesis. On the basis of the arterial – venous differences, it appears that the kidney plays a minimal role in urate synthesis, since only alanine among the potential urate precursors (Mapes & Krebs, 1978) is removed by kidney of fed chickens. This is supported by the observation by Chin & Quebbeman (1978) that approx. 80% of urate is synthesized in the liver of the fed chicken.

The starved chicken

The interorgan metabolite fluxes in the starved chicken differ markedly from those in the fed bird. The hindquarter in starvation releases glutamine, alanine, glycine and lysine, as well as lactate. Similar to the situation in mammals (Aikawa *et al.*, 1973; Felig, 1975), glutamine and alanine are released in proportions in excess of their proportion of the amino acids in muscle protein. Together glutamine and alanine account for 57% of the amino acids released by hindquarter. Alanine

alone represents 36% of the amino acids released, but only 5.6% of the amino acid content of chicken muscle (Food and Agriculture Organization, 1970). Lactate is released in amounts which provide more gluconeogenic carbon than the total of the gluconeogenic amino acids released. Glycine, an important uric acid precursor, is also released from hindquarter, but in amounts consistent with its hindquarter protein content, suggesting there is little synthesis or degradation of glycine in hindquarter.

Glycerol is released by hindquarter of starved chicken. We assume that this represents a contribution of adipose-tissue metabolism to the hindquarter arterial–venous differences. Glycerol is released from adipose tissue as part of starvation-induced lipolysis (Newsholme & Leech, 1983). The glycerol is taken up by kidney, where it is available for gluconeogenesis. Watford *et al.* (1981) have shown that glycerol is an excellent gluconeogenic substrate in kidney tubules prepared from starved chickens.

Although we found an increase in 3-methylhistidine in the blood of starved chickens, which is consistent with increased muscle protein degradation, we did not observe the release of 3-methylhistidine from the hindquarter. There are several possible explanations for this. Breast muscle in the chicken is a predominantly fast-twitch white muscle, in which the myosin contains 3-methylhistidine, in contrast with the muscle of hindquarter, which has the slow- and fast-twitch fibres. In the slow-twitch (red) fibres, myosin does not contain 3-methylhistidine, although both fibre types contain actin, which does contain 3-methylhistidine (Kuehl & Adelstein, 1970; Pollard & Weihing, 1974). Thus, if both breast and hindquarter muscle are degraded at the same rate, more 3-methylhistidine would be released from breast than from hindquarter. We did not measure an arterial–venous difference across breast muscle. Fisher *et al.* (1975) have reported the content of 3-methylhistidine in chicken breast muscle to be much higher than that found in total chicken skeletal muscle (Hayashi *et al.*, 1985), which suggests a greater contribution of breast muscle to 3-methylhistidine excretion. Given the low-whole concentration of 3-methylhistidine and the probable high blood-flow rate, the difference across the hindquarter may have been too small to be detected with our procedures. If this is so, the rise in the whole-blood concentration of 3-methylhistidine could have been the result of a small decrease in clearance of 3-methylhistidine over the 6 days of starvation in our experiment.

The kidney of starved chickens removes alanine and glycine from the circulation. Although these are uric acid precursors, it is unlikely that they are used for its synthesis, since it has been reported that urate synthesis is decreased or absent in kidney of starved fowl (Chin & Quebbeman, 1978; Martindale, 1969). It seems likely that the glycine removed is the precursor of the serine released by the kidney. Glycine has been shown to be an important precursor or serine in the rat kidney (Lowry *et al.*, 1985; Pitts *et al.*, 1963).

The kidney also extracts lactate, and, in contrast with the fed state, releases glucose. Although gluconeogenesis is readily demonstrated *in vitro* by preparations of renal tissue from a wide variety of mammals, unequivocal demonstration of this process *in vivo* has been more difficult because of the high rate of renal blood flow, together with the fact that the renal cortex is gluconeogenic, whereas the renal medulla is glycolytic. However, direct evidence of addition of glucose to renal venous blood has been obtained in the prolonged-starved obese human, where renal gluconeogenesis has been calculated to account for 45% of total glucose production (Owen *et al.*, 1969). in experimental animals, it has not always been possible to demonstrate renal gluconeogenesis directly, except in the cat, where the existence of two renal veins, one of which drains the cortical region exclusively, has permitted unequivocal demonstration of renal gluconeogenesis (Friedman & Toretti, 1978). In other experimental animals such as the rat or dog, clear evidence for renal gluconeogenesis *in vivo* has required the use of eviscerated animals (Reinecke, 1943; Reinecke & Hauser, 1948; Smith & Long, 1971) or sophisticated techniques such as the decrease in the specific radioactivity of radioactive glucose brought about by the synthesis of unlabelled glucose in the kidney (Costello *et al.*, 1973; Kida, 1978). In contrast with the difficulty of demonstrating renal gluconeogenesis *in vivo* in mammals, the data in Table 2 clearly show that this process occurs in chickens. Although it is possible that starvation changed the proportion of arterial to renal portal blood, our results are supported by the established induction of cytosolic phosphoenolpyruvate carboxykinase and the increased capacity of isolated kidney tubules to produce glucose (Craan *et al.*, 1982, 1983; Watford *et al.*, 1981). The kidney is clearly a major gluconeogenic organ in the chicken.

Ammonia was released into the renal vein in the starved chicken (Table 2), which is similar to the situation in mammals, where a fraction of intrarenally produced ammonia diffuses into tubular urine to facilitate acid excretion, and the remainder (as much as one-half) diffuses into the renal vein (Stone *et al.*, 1967). Renal ammonia production facilitates acid secretion in chickens via the same mechanism as in mammals, and indeed the urinary excretion of ammonia increases during starvation (Sykes, 1971) and the capacity for renal ammoniagenesis from alanine is increased in kidney tubules isolated from starved chickens (Watford *et al.*, 1981). We observed an increase in the uptake of both alanine and glycine by kidney of starved chickens. Ammoniagenesis from alanine increased in tubules from starved chickens (Watford *et al.*, 1981), and the synthesis of serine from two molecules of glycine also produces ammonia. The renal venous release of ammonia during starvation is therefore a reflection of increased renal ammoniagenesis and the rapid diffusion of ammonia into the renal vein by the process of non-ionic diffusion. We could not identify a tissue which removes ammonia, although it was expected that the liver would do so (Mapes & Krebs, 1978).

Carnosine (β-alanylhistidine) is removed by kidney of starved chickens. It can be hydrolysed to β-alanine and histidine by carnosinase, which is present in significant activity in the chicken kidney (Wolos & Piekarska, 1975). The β-alanine and histidine must be largely metabolized by kidney, since neither is released by the kidney. Histidine can be converted into glucose, and thus carnosine may be a source of renal glucose.

The liver of the starved bird removes serine, glutamine, alanine, glycine and lactate and releases urate and glucose. Glutamine, serine, alanine and lactate are gluconeogenic precursors in the mammalian liver (Ross *et al.*, 1967; Söling & Kleineke, 1976). However, whereas

lactate is a good gluconeogenic precursor in starved-chicken hepatocytes, alanine and serine are poorly utilized for glucose synthesis, presumably because of the lack of cytosolic phosphoenolpyruvate carboxykinase in chicken liver (Brady *et al.*, 1979; Watford *et al.*, 1981). Both alanine and serine are converted into pyruvate before conversion into glucose. Species with no cytosolic phosphoenolpyruvate carboxykinase are limited in their capacity for gluconeogenesis from pyruvate, because of a lack of the cytosolic reducing equivalents needed for gluconeogenesis. In species with a cytosolic phosphoenolpyruvate carboxykinase, cytosolic pyruvate is converted in the mitochondria into malate by pyruvate carboxylase and malate dehydrogenase. The malate leaves the mitochondria and is reoxidized to oxaloacetate by cytosolic malate dehydrogenase, thus generating a cytosolic NADH molecule for each pyruvate molecule. The oxaloacetate is converted into phosphoenolpyruvate by cytosolic phosphoenolpyruvate carboxykinase, and the necessary reducing equivalents for gluconeogenesis are available because they have been transferred from the mitochondria as malate. Without a cytosolic phosphoenolpyruvate carboxykinase, as in chicken liver, the pyruvate is converted into phosphoenolpyruvate in the mitochondria by pyruvate carboxylase and phosphoenolpyruvate carboxykinase. The phosphoenolpyruvate is then transported to the cytosol, where it is used for gluconeogenesis. However, in this case, no mitochondrial reducing equivalents are transferred to the cytosol and thus gluconeogenesis is limited. Gluconeogenesis from lactate is not affected by the location of phosphoenlypyruvate carboxykinase, because the conversion of lactate into pyruvate by lactate dehydrogenase in the cytosol generates the cytosolic NADH needed for gluconeogenesis (Williamson, 1976).

Both alanine and glutamine can provide both carbon and nitrogen for uric acid synthesis (Wiggins *et al.*, 1982; Mapes & Krebs, 1978). Although alanine was the best single amino acid substrate, alanine plus glutamine gave rates of uric acid synthesis greater than with alanine alone. Metabolism of serine via serine hydroxymethyltransferase provides methylenetetrahydrofolate needed for urate synthesis. It therefore seems likely that the glutamine, alanine and serine taken up by the liver are used for synthesis of uric acid rather than glucose. The flow of amino acids from hindquarter, in particular glutamine, alanine and glycine, thus provides both the nitrogen and carbon for uric acid synthesis and a means for transfer of gluconeogenic carbon to the liver.

The observation that branched-chain amino acids are taken up by the liver and muscle in the fed, but not in the starved, state is consistent with the model of interorgan metabolism of branched-chain amino acids in mammals (Harper, 1986). The liver takes up branched-chain amino acids in the fed state for protein synthesis and some oxidation. Oxidation is limited by low branched-chain amino acid transaminase activity in liver. Muscle, with high branched-chain amino acid transaminase activity and low branched-chain amino acid dehydrogenase activity, takes up branched-chain amino acids and converts them into the corresponding keto acids, which are released to the blood. The amino groups are transferred to alanine and glutamine, which are released to the blood, as we observed in the starved chicken (Table 2).

Although amino acids can contribute to gluconeogenesis, it should be noted that approximately two-thirds of the glucogenic carbon leaving the hindquarter leaves as lactate and that in liver and kidney approx 80% of the gluconeogenic precursors removed is accounted for by lactate. The large contribution of lactate is not surprising, since experiments *in vitro* using either isolated hepatocytes or kidney tubules have clearly demonstrated that, although both hepatocytes and tubules are capable of gluconeogenesis from amino acids as well as from lactate, the rates of gluconeogenesis are greater from lactate than from amino acids (Brady *et al.*, 1979; Craan *et al.*, 1982, 1983; Watford *et al.*, 1981). The predominance of lactate in the interorgan flux of gluconeogenic carbon makes it unlikely that the lack of cytosolic phosphoenolpyruvate carboxykinase presents a serious limitation on hepatic gluconeogenesis in the starved chicken *in vivo* (Bannister & O'Neill, 1981; Söling *et al.*, 1973; Tinker *et al.*, 1984*b*; Watford *et al.*, 1981).

Renal contribution to gluconeogenesis

From the published value for total renal blood flow of 67 ml/min per kg (Skadhauge, 1973) and our value for total liver blood flow of 36 ml/min per kg, together with the measured glucose arteriovenous differences, one can calculate that in the starved birds the liver produces 52 μmol of glucose/min per kg and the kidneys produce 21 μmol/min per kg. This total glucose production of 73 μmol/min per kg agrees very well with the glucose production rates of 56–72 μmol/min per kg determined *in vivo* starved cockerels with either [2-^{3}H]- or [6-^{3}H]-glucose injected simultaneously with [U-^{14}HC]glucose (Brady *et al.*, 1978). Thus the kidney provides 29% of the glucose in the starved chicken. Although this estimate is an approximation, it does provide support *in vivo* for the contention of Watford *et al.* (1981) that the kidney plays a substantial role in gluconeogenesis in the starved chicken.

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